Amendments to the Specification:

Please amend the specification, without prejudice, by substituting the following heading and paragraphs:

On page 9, line 4:

Please substitute the following heading: ~~BRIEF~~ DETAILED DESCRIPTION OF THE ~~DRAWINGS~~ INVENTION.

On page 9, lines 6-7:

Please amend as follows:

The invention relates to a non-human transgenic ~~milk that can be used to~~ animal that produces therapeutical recombinant hFVIII in the milk of the non-human transgenic animal for treating hemophilia ~~patent treatment~~ patients.

On page 9, lines 9-20:

Please amend as follows:

Using mammary gland-specific promoters, a wide range of proteins of bio-pharmaceutical interest have been expressed in rodents, pigs, and dairy animals (Echeland, 1996, Current Opinion in Biotechnology 7: 536; Houdebine et al., 2000, Transgenic Research 9:505). An expression vector, comprising a gene encoding the target protein of interest fused to a milk promoter gene, is introduced by microinjection into the pronucleus of a one-cell embryo. Upon germ line integration and expression, the transgene ~~becomes~~ acquires a dominant Mendelian genetic characteristic that is inherited by the progeny of the founder animal. ~~Mammalian mammary~~ Mammary epithelial cells of the non-human transgenic animal have the capacity to carry out complex protein synthesis with a variety of posttranslational modifications and folding.

From page 10, line 22, to page 11, line 4:

Please amend as follows:

The recombinant gene construction can also include ~~a~~ signal sequences, particularly ~~a~~ signal peptide sequences of a milk specific gene. ~~For the best secretion efficient of forigen~~To achieve optimal secretion of foreign protein ~~in~~ by the mammary gland, the milk-specific signal

peptide sequence, ~~can be the~~ such as a secretional signal sequence which naturally occurs with the selected milk-specific promoter, can be used in the construct~~, which are described below~~. For example, signal sequences from genes coding for caseins, e.g., alpha, beta, gamma or kappa caseins, beta lactoglobulin, whey acid protein, and lactalbumin can be used.

On page 11, lines 6-14:

Please amend as follows:

A 2.0-kb promoter sequence of bovine alpha-lactalbumin (αLA) was generated by PCR amplification using a genomic DNA from high milk-producing Holstein cow, which obtained from National Taiwan University Farm, as the template. This PCR product ~~containing~~ contains an entire αLA promoter and a 19-aa leader sequence ~~(SEQ ID: No. 1)~~ encoded by a DNA sequence of SEQ ID NO: 1 or replacement with a 15-aa bovine αS1-casein ~~15-aa~~ signal peptide sequence ~~(SEQ ID: No. 2) which created~~ encoded by another DNA sequence of SEQ ID NO: 2, as well as a restriction enzyme~~, HpaI,~~ (HpaI) cloning site ~~in the~~ created downstream of the DNA sequence of SEQ ID NO: 1 or SEQ ID NO: 2. ~~These two types of~~ The promoter and signal peptide sequences were then subsequently inserted into the pCR3 vector (Invitrogene, San Diego, CA).

From page 11, line 16, to page 12, line1:

Please amend as follows:

For the full-length hFVIII construction, ~~the resulting~~ a 7.0-kb resultant plasmid containing αLA promoter and its intact signal peptide sequence was double digested with MluI and PstI and treated with calf intestinal phosphatase (Boehringer Mannheim, Indianapolis, IN). The pCMV5/hFVIII plasmid containing the intact human FVIII coding sequence was also double digested with MluI and PstI. The in-frame sequence from the αLA leader peptide through the hFVIII junction was determined using the Dye Terminator sequencing system (Applied Biosystems Inc., Foster, CA). The 9.7-kb transgene consisting of 2.0-kb bovine αLA promoter, 7.2-kb hFVIII cDNA, and 0.5-kb bovine GH gene polyadenosine signal sequence was separated from plasmid pCR-.alpha.LA/hFVIII-5 using ClaI and XbaI digestion and purified ~~for~~

~~microinjection on the~~ by ultra-centrifugation twice ~~$CsCl_2$ ultra-centrifugation~~ through a $CsCl_2$ gradient before microinjection.

On page 12, lines 2-21:

Please amend as follows:

For the B domain-deleted hFVIII construction, the ~~resulting~~ 7.0-kb resultant plasmid containing αLA promoter and αS1-casein signal peptide sequence was double digested with HpaI and XhoI and treated with calf intestinal phosphatase (Boehringer Mannheim, Indianapolis, IN). The pCMV5/hFVIII plasmid containing the intact human FVIII coding sequence was used as a template to generate a 2233-bp rhFVIII A-domain fragment ~~using~~ by degenerate PCR, using a pair of primers pHFVIII-HpaI(+): 5'-GGT TAA CTG CCA CCA GAA GAT A-3' ~~(SEQ ID: NO.3)~~ (SEQ ID NO: 3) and phFVIII-741aa(-): 5'-AAG CTT CTT GGT TCA ATG GC-3' ~~(SEQ ID: NO.4)~~(SEQ ID NO: 4), and a 2085-bp rhFVIII C-domain fragment amplified by a pair of primers pHFVIII-1643aa(+): 5'-AAG CTT GAA ACG CCA TCA ACG GGA A-3' ~~(SEQ ID: NO.5)~~ (SEQ ID NO: 5) and phFVIII-XhoI(-): 5'-CTC GAG CCT CAG TAG AGG TCC TGT-3' ~~(SEQ ID: NO.6)~~ (SEQ ID NO:6), respectively. ~~Equal molar ratio of~~ A-domain segment, C-domain segment and pCR3-αLA vector of equal molar ratio were co-ligated and transformed into host competent cells. The in-frame sequence from the αS1-casein leader peptide through the hVIII junction was determined using Dye terminator sequencing system (Applied Biosystems Inc., Foster, CA). The 6.8-kb transgene consisting of 2.0-kb bovine αLA promoter and ~~aS1~~ αS1-casein leader sequence, 4.3-kb hFVIII cDNA, and 0.5-kb bovine GH gene polyadenosine signal sequence was separated from plasmid pCR-αLA/hFVIII(ΔB) using ClaI and XbaI digestion and purified ~~for microinjection on the twice $CsCl_2$~~ by ultra-centrifugation twice though a $CsCl_2$ gradient ~~ultra-centrifugation~~ before microinjection.

From page 12, line 23, to page 13, line 21:

Please amend as follows:

The purified transgene was microinjected into the male pronuclei of fertilized eggs from superovulated female mice of the outbreed ICR strain and transferred to recipient pseudo-pregnant females as previously described (Chen et al., Transgenic Res 4: 52-59; 1995). For

transgenic goat production, the pronuclear stage embryos were flushed from the donor goat's oviduct at the one and a half day after insemination by means of a surgical method. In order to obtain ~~higher embryo numbers~~ a larger number of embryos, every embryo-donating goat had been treated with endocrine ~~so as~~ to achieve ~~the object of~~ superovulation. ~~Such a~~ The superovulation treatment ~~comprised of administrating~~ involves intramuscular administration of follicular stimulating hormone (FSH) to the embryo-donating goat ~~sequentially~~ twice a day with an interval of 12 hours for 4 consecutive days ~~since~~ from the eighth day of the estrous cycle ~~twice a day with interval of 12 hours~~ and the dosage was gradually decreased daily as 4-, 3-, 2-, and 1-mg, respectively. ~~As the first dosage at~~ On the third day, the first dosage of FSH was co-administrated with 1000iu human chorionic gonadotropin (HCG) which resulted in detection of ~~estrous after~~ estrogen 54 hours ~~whereupon gave~~ after two artificial ~~insemination~~ inseminations (AI) were given with an interval of 12 hours. Then, at about one and a half days after conception, the ~~goat~~ one-cell stage goat embryos were collected with a sterile glass capillary tube via a surgical embryo flushing method. The collected embryo was transferred into another petri dish ~~where it was~~ and rinsed more than ten times. Thereafter, ~~it~~ the collected embryo was placed under a phase contrast microscope at 400X amplification for gene microinjection manipulation. After a transient in vitro culture, the healthy microinjected embryos were than transferred into recipient oviducts for further ~~conceptus~~ conception development.

From page 13, line 21, to page 14, line 12:

Please amend as follows:

The resulting pups were rapidly screened for the transgene by PCR amplification of tail or ear tissue DNA. PCR was performed using one set of ~~primer~~ primers, pαLA-124(+): 5'-CTC TCT TGT CAT CCT CTT CC-3' ~~(SEQ ID: NO.7)~~ (SEQ ID NO: 7) and phFVIII-149(-): 5'-GGT TAC GCG TCA AGA TTC TGA-3' ~~(SEQ ID: NO.8)~~ (SEQ ID NO: 8), which defined a 273-bp region spanning the αLA promoter, secretion signal sequence and hFVIII cDNA junctional sequence. For the B domain-deleted hFVIII transgene detection, additional primer pairs ~~was~~ were designed, phFVIII-ACJ(+): 5'-AGA CTT TCG GAA CAG AGG CA-3' ~~(SEQ ID: NO.9)~~ (SEQ ID NO: 9) and phFVIII-ACJ(-): 5'-ATC TTT TTC CAG GTC AAC ATC A-3' ~~(SEQ ID: NO.10)~~ (SEQ ID NO: 10), which defined a 751-bp region flanking A-C recombinant junction.

The positive PCR screening results for transgenic animals were further confirmed by Southern blot analysis. Ten micrograms of genomic DNA were individurally digested with BamHI, HindIII, PvuII, and XbaI restriction enzymes at 37°C overnight, electrophoresed on a 0.8% agarose gel, and transferred to a Durose membrane (Stratagene, La Jolla, CA). A KpnI fragment of hFVIII-specific cDNA (1.8-kb) was used as a radioactive probe to hybridize the membrane. Blots were subjected to autoradiography for three days at -20°C.

On page 14, lines 16-25:

Please amend as follows:

The temporal and spatial expression of hFVIII RNA in transgenic animals was analyzed using a reverse transcription-polymerase chain reaction (RT-PCR). Total RNA from different tissues including the heart, liver, lung, muscle, mammary gland, brain, ~~pancrease~~ pancreas, and kidney of female transgenic mice during lactation periods (Day 1 to Day 28 post partum) was extracted using the acid guanidinium thiocyanate method (Chomczynski and Sacchi, 1987, Anal. Biochem. 162: 156-159). One microgram of total RNA was treated twice with 10 units of DNase (Gibco BRL, Gaithersburg, MD) and phenol-chloroform ~~extracted~~ extract. RNA pellets were resuspended in 15 µl DEPC-water and then used to synthesize the first-strand cDNA with random primers and SuperScript reverse transcriptase (Gibco BRL, Gaithersburg, MD) in a total volume of 25 ml. The reaction was carried out at 42°C for 1 hr.

On page 15, lines 1-9:

Please amend as follows:

For further PCR amplification, an aliquot (1/10) of the RT product was adjusted to contain 0.1 µg of each primer and additional buffer was added for a total volume of 50 µl. PCR was performed for 30 cycles (94°C., 1 min; 55°C, 2 min; 72°C, 2 min). The primers used included a pair of hFVIII-specific primers, phFVIII-F2(+): 5'-CAT TCT ATT CAT TTC AGT GGA CA-3' ~~(SEQ ID: NO.11)~~ (SEQ ID NO: 11) and phFVIII-R2(-): 5'-GAG ATG TAG AGG CTG GAG AAC T-3' ~~(SEQ ID: NO.12)~~ (SEQ ID NO: 12), and a pair of glyceraldehydes-3-phosphate dehydrogenase (GAPDH), as well as a pair of β-actin. Both β-actin and GAPDH are the universal transcripts in every cell and were used as the internal controls of RT-PCR.

From page 15, line 11, to page 16, line 3:

Please amend as follows:

Milk was collected from lactating females as previously described (Simons et al., 1987, ~~Nature 328: 530-532.)~~Nature 328: 530-532) and analyzed using SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (Cheng et al., 1998, Human Gene Therapy 9: 1995-2003). Ten- to twenty-fold dilutions of the collected milk, in 75 mM Tris-HCl buffer at pH 6.8, from different lactation periods were diluted in SDS-PAGE sample buffer with 5% 2-mercaptoethanol and electrophoresed on 7.5% gel. To estimate production levels, HPLC-purified recombinant hFVIII standard was diluted to 10 μg/ml in normal mouse milk and electrophoresed alongside the ~~transgenic~~ milk samples from the transgenic animals. Proteins were ~~electrotransferred~~ transferred from the gel to a PVDF membrane (NEN Life Science products, Boston, MA). The protein blots were probed with primary antibodies ~~recognizing~~ binding to hFVIII at 2 to 10 μg/ml and washed with phosphate-buffered saline containing 0.1% Tween-20 (PBS-T). ~~Blots~~ The protein blots were further reacted with horseradish peroxidase (HRP)-conjugated ~~second~~ secondary antibodies at 0.2 mg/ml. ~~Polyclone~~ The polyclonal antibody C6 ~~recognize~~ binds to the heavy chain of hFVIII ~~rang~~ and ranged from 80-200 kDa and the monoclonal ~~monoclone~~ antibody D2 ~~recognize~~ binds to the light chain of hFVIII at 75-80 kDa. The blot was then developed with chemiluminescent ~~ECLTM~~ (ECL™) detection system (Amersham, UK) and ~~exposure to~~ exposed on a x-ray film. Band intensities were compared by densitometry.

On page 16, lines 4- 23:

Please amend as follows:

Secretion of hFVIII protein from the ~~transgenic~~ milk of the transgenic animal was determined quantitatively with enzyme-linked immunosorbent assay (ELISA) as previously described (Chen et al., 1993, J Virol 67: 2142-2148). Briefly, plates were coated with capture antibodies (ESH-5 and ESH-8, 500 ng each per well; American Diagnostics, Greenwich, CT) in carbonate buffer and incubated for 1 hour at 37°C. Plates were washed with 0.05% Tween-20 in phosphate-buffered saline (PBS) and blocked in 50 mM Tris (pH 7.2), 150 mM NaCl, 0.5% gelatin, and 0.05% Tween-20 for 2 hour at 37°C. Purified recombinant FVIII (Hyland, Baxter

Healthcare, CA), prepared in blocking buffer with 1:50 normal murine milk, served as the standard. Samples and standards were incubated for 1 hour at 37°C. Detection antibody (rabbit anti-human FVIII 1:10,000 dilution) was added and allowed to react with hFVIII at 37°C for 1 h. After ~~removing the first antibody using~~ non-specific binding of primary antibody was removed by four washes with PBS, a ~~second~~ secondary antibody, goat anti-rabbit immunoglobulin antibody conjugated with horseradish peroxidase, was added (1 : 3,000 dilution) and incubated for ~~a further~~ another hour ~~1 hour of incubation~~. The plates were washed again thoroughly with PBS, and 100 µl of substrate solution (2 mg of 0-phenylenediamine dissolve in 1 ml of 1M phosphate citrate plus 0.02% H_2O_2) was added for development. After 30 min, 100 µl of 1M H_2SO_4 was added immediately to stop the reaction and the colors were measured by optical density at 492 nm.

From page 16, line 25, to page 18, line 1:

Please amend as follows:

Expression of rFVIII protein in the milk of transgenic animals driven by bovine α-lactalbuimn regulatory sequence seems more efficient than the other transgene constructions, which were controlled by ~~ovine~~ bovine β-lactoglobulin promoter (Niemann et al., 1999, Transgenic ~~Res 8: 237-247.)~~ Res 8: 237-247) as well as mouse WAP regulatory sequence (Paleyanda et al., 1997, Nature Biotech 15: 971-975). Expression of the bovine αLA gene has been shown to be the most lactation-specific of all bovine milk protein genes (Goodman and Schanbacher, 1991, Biochem Biophys Res Commun 180:75-84). Use of the αLA 19-aa ~~secretary~~ secretory peptide ~~(SEQ ID: NO: 13)~~ (SEQ ID NO: 13) or αS1-casein 15-aa signal peptide ~~(SEQ ID: NO: 14)~~ (SEQ ID NO: 14) to lead the rFVIII protein secretion and the bGH polyadenosylation signal to stabilize the steady-state of hFVIII RNA molecules~~, which~~ may also have contributed to our success. ~~Actually, there are several~~ Several previous reports have demonstrated that mammary specific transgenes driven by αLA promoters have resulted in high levels of gene expression up to 3.7 mg/ml (Hochi et al., 1992, Mol Reprod Dev 33: 160-164; Soulier et al., 1992, Fed Eur Biol Soc Lett 297: 13-20; Maschio et al., 1991, Biochem J 275:459-465) in ~~their milks~~ the milk of the transgenic animal. This characteristic of the bovine αLA gene makes its regulatory elements potentially useful as a mammary expression system in transgenic

animals. In contrast to the caseins and ~~b~~ β-lactoglobulin, the production of αLA mRNA increases suddenly at paturition, remains elevated during lactation, and drops sharply at dry-off and during involution. The rFVIII protein profile ~~lead~~ led by the αLA promoter and 19-aa secretion signal peptide or αS1-casein 15-aa signal peptide follows a similar trend. The lactation specificity of the regulatory regions used to control mammary expression in transgenic animals may be important when foreign proteins ~~that have~~ having biological activities are expressed ~~because~~ since these proteins may ~~exhibit~~ exert their biological ~~function in~~ activities on the animals if they are secreted before tight junctions of mammary epithelial cells are formed.

From page 18, line 3, to page 19, line 5:

Please amend as follows:

Clotting factor IX (FIX), adenosine deaminase (ADA), and other cDNAs were expressed at high levels from retroviral vectors in rat fibroblasts (Miller, 1992, Nature 357: 455-460; Palmer et al., 1991, Proc Natl Acad Sci USA 88: 1330-13334), whereas the FVIII cDNA was expressed at very low levels from these vectors in primary human fibroblast cells (Hoeben et al., 1990, J Biol Chem 265: 7318-7323; Lynch et al., 1993, Hum Gene Ther 4: 259-272). It has been found that FVIII RNA steady-state levels were reduced 100-fold from a FVIII retroviral vector compared to the same vector expressing other cDNAs, and FVIII vector titers were correspondingly reduced 100-fold compared to other vectors (Lynch et-al., 1993, Hum Gene Ther 4: 259-272). A large part of the inhibitory effect of the FVIII cDNA on expression from a retroviral vector has been localized to a 1.2-kb fragment, derived from the A2 and A3 domains, which decreased the steady-state RNA levels 100- to 200-fold and decreased vector titers 10-fold (Koeberl et al., 1995, Hum Gene Ther 6: 469-479). The mechanism by which the FVIII cDNA inhibitory sequence (INS) decreases RNA and protein expression from expression vectors ~~remain~~ remains to be established. Recently, experimental data provided ample evidence that the human FVIII cDNA contains elements that repress its own expression at the level of transcription (Hoeben et al., 1995, Blood 85: 2447-2454). Fallaux and coworkers (1996, Mol Cell Biol 16: 4264-4272) identified a 305-bp region derived from exons 9 to 11 that encodes a nuclear-matrix attachment region (MAR), also called the scaffold-attached region (SAR). Yeast MARs elements can play an important role in transcriptional silencing (Newlon and Theis, 1993,

Transgenic Res 8: 237-247). B-domain-deleted hFVIII cDNA (BDD-hFVIII) is a 4.3 kb ~~and~~ sized sequence that encodes a primary truncate polypeptide (hFVIIIΔB) ~~is 1448 aa (SEQ ID: NO. 15)~~ of (SEQ ID NO: 15) not previously thought feasible for testing in transgenic animals ~~previously~~.

From page 19, line 13, to page 20, line 12:

Please amend as follows:

Out of 79 potential transgenic founder mice, 17 were identified as being transgenic by the PCR screening (Fig. 1B). Two transgenic founder goats carrying the full-length hFIII also have been identified by PCR screening (Fig. 1C) as well as two lines of transgenic pigs have been established. To quantify the transgene copy numbers and to understand the integration patterns of the foreign gene within the genome the transgenic animals, restriction enzyme BamHI was used to digest the genomic DNA, cut once within the transgene, and subjected to Southern blotting analysis. On hybridization using the 1.8-kb KpnI fragment of human-specific FVIII sequence as a probe (Fig. 1A), a 9.7-kb band representing monomeric transgene copies present in head-to-tail joining (H-T) tandem repeats was observed in all cases. A 4.6-kb hybridization band was observed in the blots of all transgenic mice present in tail-to-tail joining (T-T) inverted repeats (Fig. 1D). Different intensities of the hybridization signal were also observed indicating the presence of different transgene copy numbers in these mice. ~~Further detail quantification using~~ A slot-blot hybridization was further carried out for detailed quantification (Fig. 2A ~~and~~ 2B) of the hybridization signal, ~~they~~ which could be ~~distinguished~~ classified into three groups according to the copies of transgene integration. For low copy number (defined as 1-5 copies per cell), there were six transgenic lines including αLAF-7, -15, -33, -36, -43 and -54. Transgenic founders carrying middle copy number (10-20 copies per cell) were found in nine lines including αLAF-9, -13, -18, -27, -28, -29, -30, -38 and -39. In addition, there were two lines αLAF-8 and -25) carrying high copy number (40-50 copies per cell) of hFVIII transgene in their genome. All of the transgenic mice harboring αLA-hFVIII DNAs presented two-to-three off-size bands (Fig. 1D) besides the predicted transgene junctions. These bands were probably rearranged transgene sequences, some of which were integration sites representing transgene-cellular junctions.

On page 20, line 13-17:

Please amend as follows:

Breeding lines are established from all transgenic founder animals by crossing them with normal ICR mice, Alpine goats, or ~~hybride~~ hybrid pigs. The multiple integrated copies of the transgene were found to be stably germ-line transmitted among twelve (7 females and 5 males) out of the 17 transgenic founders.

From page 20, line 25, to page 21, line 3:

Please amend as follows:

In the middle of rFVIII polypeptide, the A-domain (741-residue) has been created to join the c-domain (1643-residue) for completely ~~deleted~~ deleting the B-domain segment (Fig. 3C).

On page 21, lines 6-13:

Please amend as follows:

Out of 65 potential transgenic founder mice, 15 were identified as being transgenic by the two sets of primers, with one pair of ~~primer~~ primers located in the 5'-promoter junction, and the other pair of ~~primer~~ primers flanking the B-domain deletion region (Fig. 4A). The PCR screening was shown in Fig. 4B. To quantify the transgene copy numbers and to understand the integration patterns of the foreign gene within the genome of the transgenic mice, restriction enzyme HindIII, PvuII, and XbaI were used to digest the genomic DNA, cut once within the transgene, and subjected to Southern blotting analysis (Fig. 4C). The result showed that transgenic goat (Tg-3431) harboring B-domain-deleted hFVIII fusion gene clearly exhibited one to two off-size bands in their genome when ~~compairing~~ compared with normal goat genome.

On page 21, lines 14-18:

Please amend as follows:

Germ-line transmission ~~ability~~ of ~~transgenic animals carrying~~ hFVIII(ΔB) exogenic DNA ~~in~~ from transgenic animals to their F1 generation was confirmed by using PCR detection and direct PCR product sequencing. The sequencing result exhibited the transgenic hFVIII(ΔB)

fragment was actually present in ~~their~~ the F1 ~~offspring's~~ offspring genome of transgenic goats (Fig. 5).

From page 21, line 23, to page 22, line 14:

Please amend as follows:

To characterize the spatial expression of the new developed mammary-specific expression cassette containing hFVIII in this invention, total tissue RNAs were extracted from different organs in the breeding lines established from the transgenic founder mice. Reverse-transcriptional polymerase chain reaction (RT-PCR) analysis was performed using two pair of primer sets, one was phFVIII-F2(+) ~~(SEQ ID: NO.11)~~ (SEQ ID NO:11) and phFVIII-R2(-) ~~(SEQ ID: NO.12)~~ (SEQ ID NO: 12) specific to human FVIII cDNA franking 3'-coding region, the other was ~~b-actin~~ β-actin primer set as an internal control. As shown in Fig. 6A, the transgene expressed transcriptant of the 410-bp hFVIII RT-PCR product was found in the mammary gland of lactating transgenic mouse. No homologous transcripts were detectable in the heart (H), liver (L), lung (LU), muscle (M), brain (B), prancreas (P), or in male transgenic mouse mammary fat pads (F). All examined tissues showed a 420-bp amplified fragment for mouse ~~b-actin~~ β-actin to assess the integrity and quantity of RNA in each sample. The efficiency of DNase I treatment to eliminate DNA contamination was determined using RNA from a transgenic mammary gland. When the reverse transcriptase was omitted from the reaction, no amplification was observed (Fig. 6A, last lane).

From page 22, line 25, to page 23, line 7:

Please amend as follows:

To quantify the level of hFVIII mRNA in the mammary glands of transgenic mice, densitometric analysis was performed~~,~~ for comparing the intensity of the 250-bp band in each lane normalized to the level of signal generated upon co-amplification of the mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers. The intensity of the hFVIII transcript increased eight-fold and ten-fold upon Day 8 and Day 15 post-parturition, respectively, ~~while a~~ whereas the intensity was decreased by two-fold ~~decrease was present~~ on Day 22 of the later lactation (Fig. 6B)~~,~~ when compared to Day 1 of the newly lactating stage.

From page 23, line 10 to page 24, line 2:

Please amend as follows:

Expression and secretion of recombinant hFVIII proteins into the ~~transgenic milks~~ milk of transgenic animals

To determine the production of recombinant hFVIII and to compare the secretion efficiency of rFVIII among ~~the~~ different transgenic mouse lines, milk was collected from five lines of F0, F1 and F2 females during lactation. Proteins from skim milk of both non-transgenic control animals and transgenic ~~skim milks~~ animals were separated in SDS-PAGE and followed by Western blot analysis (Fig. 7A ~~–~~ and 7B). No polypeptides characteristic of recombinant hFVIII were detected in control mouse milk using the hFVIII-specific polyclonal antibody. In ~~transgenic~~ the milk of transgenic animals, rFVIII was detected as a ~~heterogenous~~ heterogeneous group of polypeptides of approximately 80-200 kDa, of which the 92-200 kDa polypeptides represent the heavy chain and its proteolytic cleavage products of different glycosylational modification patterns. The 80 kDa small molecule ~~of an 80 kDa corresponding~~ which corresponded to the light chain containing hFVIII C2 domain was also detected by this antibody (Fig. 7A). ~~In similar~~ Similar experiments were conducted using monoclonal antibodies that ~~recognize~~ bind to specific hFVIII light chain C2 domain structures, and a specific polypeptide corresponding ~~in size~~ to ~~the~~ a 80 kDa size was observed ~~as~~ and compared with the HPLC-purified hVFIII C2 domain peptide (Fig. 7B).

On page 24, lines 12-13:

Please amend the heading as follows:

Biological function assay for clotting activity of recombinant hFVIII derived from the~~transgenic milks~~ milk of transgenic animals

On page 25, lines 1-9:

Please amend as follows:

The concentrations of rFVIII ranged from 7.0 to 50.2 μg/ml, over 35- to 200-fold higher than that in normal human plasma (Table 1). The expression levels of rFVIII from three groups

~~on different line~~ carrying low, middle and high copy numbers of transgenes were not significantly different. Recombinant hFVIII production generally tended to increase through the lactation period, but dropped dramatically at the end of lactation. This result was in parallel with the RNA transcript assay (FIG. 6A~~—~~and 6B) as well as the typical mouse milk production curve, mouse milk yield increased throughout

On page 25, lines 10-18:

Please amend as follows:

The functional activity of rFVIII was examined using one-stage clotting assay~~s~~ (Table 1) ~~and this approach demonstrated~~ to demonstrate that rFVIII secreted in the milk of transgenic mice was biologically active. In the clotting assay, the addition of transgenic mouse milk~~, but not control milk,~~ to FVIII-deficient human plasma resulted in the restoration of normal coagulant activity. Up to 13.41 U/ml of rFVIII procoagulant activity was detected. The activity of rFVIII was detected throughout lactation as shown in Table 1. The same result for rFVIII clotting activity derived from transgenic goat also has been demonstrated and ~~showed~~ shown in FIG. 9A~~—~~to 9C.

On page 27, lines 17-19:

Please amend as follows:

4. Biological active recombinant hFVIII protein derived from ~~transgenic milks~~ the milk of transgenic animals is easily collected by a diary automatic milk collection system and ~~simply~~ purified ~~procedure~~ to obtain the massive recombinant ~~proteins~~ protein.

Amendments to Sequence Listing

Please substitute the amended sequences of SEQ ID NOs: 1 - 15 as indicated in an Amended Sequence Listing submitted herewith as pages 28-43 for the previously amended sequences filed on August 30, 2004, which in turn replaced the original Sequence Listing at pages 28-43 of the application as filed. The present Amended Sequence Listing therefore is identical to the original Sequence Listing of the application as filed.